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Hyperplastic Epidermal Disease in the Bluegill Sunfish, *Lepomis macrochirus* Rafinesque.

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(Plates I-VII).

INTRODUCTION.

Hyperplastic epidermal disease has been reported frequently among European freshwater cyprinids, but only rarely from other species in this and other parts of the world. This disease, variously called carp-pox, Pockenkrankheit des Karpfens, varioles des carps, cutaneous warts and epithelioma papillosum, has been known since the Middle Ages. It is characterized by white or grayish, irregular, raised patches scattered over the skin. Histologically there is a striking hyperplasia of epithelial cells and other epidermal elements, supported by a delicate stroma. According to Hofer (1904), the first accurate description probably was given by Wierzejski in 1887. This atypical growth was restudied and recorded from carp and related fishes by the following: Hofer (1896 a, b; 1901; 1904), Doflein (1898, 1928), Plehn (1906, 1924), Loewenthal (1907), Fiebiger (1909), Thomas (1931) and Schäperclaus (1935).

A somewhat similar hyperplastic disease has been recorded for marine fishes. Johnstone (1925) reported skin lesions in dabs and plaice taken off the coast of England. He referred to these lesions as cutaneous warts or epitheliomata. Smith (1935) described a hyperplastic epidermal disease in winter flounder (*Pseudopleuronectes americanus*) caught in Long Island Sound. This was the first report of such a disease occurring in fishes of American waters.

The present report, insofar as known, is the first description of an epidermal hyperplasia in a freshwater fish of North America. The disease was found in bluegill sunfish, *Lepomis machrochirus* Rafinesque, taken from a lake near New Preston, Connecticut.

The writer is indebted to the late Mr. L. Beeman, former owner of the Warmaug Black Bass Hatchery, at New Preston, for collecting the fish; to George M. Smith, Yale Medical School, for some of the histological preparations, photographs and suggestions incorporated in this paper, and to Mr. James Atz, of the New York Aquarium staff, for criticism in the preparation of this paper.

MATERIAL AND METHODS.

The hyperplastic epidermal growths were found in bluegill sunfish for four consecutive years from 1938 to 1941. During this period about 200 diseased fish were examined. What percentage of the total population this number represented was not determined. All of the fish studied were sexually mature, measuring from 13 cm. to 24 cm. in standard length. Of this diseased group, 75% were females and 25% were males. Most of the fish were collected during the spring, at which time the epidermal growths were most extensive. Fish caught during this period were kept in aquaria and observed for over a year. They showed no further extension of the growths; instead there was a tendency for the hyperplasia to regress.

The fish were collected and stored in the pools of the Warmaug Black Bass Hatchery. Some were sacrificed, fixed and preserved in 10% neutral formalin. Others were shipped alive and placed in reserve tanks of the New York Aquarium at Battery Park, where the progress of the disease was studied. Skin lesions and various organs of the body were prepared for histological examination from preserved material. They were blocked in paraffin, sectioned at 3 and 6 microns and stained with hematoxylin-eosin and by Masson's, Giemsa's and Willhite's methods. Some scales with the growth were stained and mounted *in toto*.

Attempts at transmission were made, but in all cases negative results were obtained. Diseased fish were kept in tanks with normal-appearing bluegills and other fishes from the same area and with bluegills taken from a pond near Red Bank, New Jersey, for more than a year and a half. The New Jersey fish remained normal throughout the entire period of exposure. Dermal and intraperitoneal transplants of fresh and glycerinated material had no effect. Bits of the epidermal growth were transplanted into the anterior chamber of the eye of both normal and diseased bluegills. The tissue remained intact for several weeks but eventually was absorbed. No regeneration of the growth took place in regions where scales with the overgrowths were removed.

DESCRIPTION OF THE HYPERPLASIA.

The epidermal hyperplasia is quite similar to the disease described from other fishes.

The disease appears as whitish papillo-matous-like nodules or mucoid overgrowths on the skin of any part of the body (Figs. 1 and 3), but more frequently on the dorso-lateral surface (Fig. 2). They vary in size from that of a pin-point to 2-3 cm. in length and about 2-4 mm. in thickness (Figs. 3, 7 and 8). Some of the nodules are black or grayish in appearance due to the presence of melanophores (Fig. 5). The latter are not especially increased in amount.

The whole mounts of scales (Fig. 5) also show numerous needle-like crystals usually radially arranged and superficially placed. They measure from 10-25 microns in length, and are not alcohol or xylol soluble, nor do they stain with hematoxylin-eosin. Rod-shaped magnesium phosphate crystals were reported by Johnstone (1925) as impreg-nating the cutaneous warts of a plaice. The significance of these crystals in the epidermal hyperplasia of the sunfish and those found in the plaice is not known.

Histologically, the growth shows a great hyperplasia of epithelium without keratin-ization (Figs. 6-10). The hyperplasia appears to arise from the epithelium lining the scale pockets (Fig. 8). Here the cells may vary from the normal three or four layers to many layers as they grow over the scales (Figs. 4 and 7). The proliferating cells seem to follow the paths formed by the scale rings (Fig. 5). The hyperplasia consists of closely packed epithelial cells and epidermal ele-ments around a central core originating from the corium (Figs. 6-8). Melanophores, capil-laries, nerve elements and fibrous material are found in this core. In some areas the corium is edematous (Fig. 7) but no inflam-matory reaction is evident.

The cells in the normal regions of the epi-dermis are typically flattened epithelial ele-ments with mucus and sensory cells inter-spersed. As the cells proliferate along the surface of the scale they take on a more or less spindle shape (Figs. 5 and 9). They are eventually forced up into folds (Figs. 7 and 8), with the corial tissue growing into them (Fig. 8). Along these folds the epithelial cells are columnar with a somewhat thick-ened basement membrane (Figs. 7, 8 and 10). These cells form the basal elements of the hyperplasia. The outermost cells take on a polyhedral and squamous appearance, and seem to be connected with each other by intercellular bridges, which probably ac-counts for the rigidity of the growth (Fig. 10). In hematoxylin-eosin and Giemsa's preparations the cells stain homogeneously, each with a typically appearing nucleus. No nuclear or cytoplasmic inclusions are seen in these or in Willhite preparations made for this purpose. Pro-chromosomes are evident in many of the nuclei (Figs. 9 and 10), and typical mitotic figures occur frequently (Fig. 10). Numerous mucus cells are present (Fig. 8). These are more prominent around the periphery but they may be clustered in any area of the growing tissue. Occasionally they are arranged around a central lumen forming a follicle-like structure (Fig. 11). The secre-tion within the lumen stains pink or yellow-ish with Giemsa's and green with Masson's. Sensory cells in the epithelium are not in-volved in the hyperplasia.

Fishes caught later in the year show many regressive changes. The orderly formation of cell layers, as seen in Figs. 6 and 10, gives way to a disorganized growth. Many of the epithelial cells appear oval in shape with scanty or vacuolated cytoplasm, pyknotic nuclei and with karyorrhexis evident here and there. With Masson's, the tissue gives a metachromatic picture. There is a tendency for the peripheral cells to slough (Fig. 14), and those in the interior of the growth to form epithelial cyst-like bodies (Fig. 12). In some areas there is an abortive attempt to regenerate as evidenced by the presence of cells undergoing mitosis in regions showing considerable degeneration. Eventually, the hyperplastic tissue is invaded by macro-phages and degeneration becomes more ex-tensive.

The ecto-parasitic ciliates, *Trichodina* and *Ichthyophthirius*, are found associated with the disease in every preparation studied. The *Ichthyophthirius* is encysted in the growth, while *Trichodina* is found free on the surface (Figs. 13 and 14).

There is no evidence of invasion into the sub-dermal region, nor is there any evidence of metastatic growth. Sections of the various internal organs appear normal. The indica-tions are, then, that the hyperplastic epi-dermal disease of the bluegill sunfish, as in other freshwater and marine fishes, is a benign regressive disease.

DISCUSSION.

Epidemiologically, the epidermal hyper-plastic disease in cyprinids and in the blue-gill sunfish gives evidence that an infectious process may be involved. The occurrence of the hyperplasia in the same species of fish, and in the same pond or lake year after year would point in that direction. In the Euro-pean fish, the disease is usually endemic dur-ing the warmer months. In certain years epidemics have been reported throughout the continent, but more often it occurs only sporadically. In one lake, Jasmunder Bodden, in northwest Germany, 20% of the cyprinids (carps, ides, breams, etc.) collected during the summer were found affected (Plehn, 1924). In the bluegill sunfish, the disease was found more commonly during the spring. Fish caught in this period showed more ex-tensive growths than those collected later in the year. In addition, stained sections of material collected in the spring presented more mitotic figures than that prepared from material collected in the summer. The hyper-

plastic growths in the latter group showed considerable degeneration and regression.

The exact cause of the hyperplasia, however, still remains unknown. It is interesting to report that in every instance a parasite has been found associated with the disease. Thus Hofer (1904) and Doflein (1898, 1928) recorded the presence of a histozoic myxosporidian, *Myxobolus cyprini*, and inferred that this organism might have been responsible for the hyperplasia, even though the protozoan infection was localized in the kidney, liver and spleen. Related cnidosporidians (myxosporidians and microsporidians) associated with tissue hyperplasia in other fishes have been demonstrated by several investigators: Kudo (1919, 1924), Nigrelli and Smith (1938, 1940), Nigrelli (1948). As pointed out by the latter, "Intercellular and intracellular cnidosporidians elaborate proteolytic enzymes and other chemical substances which may be responsible for considerable cellular degeneration, cell hypertrophy and other tissue responses noted in these infections. It is altogether possible that chemical substances elaborated by the developing parasites may have a stimulating growth factor which may diffuse to more distant areas either directly or through the circulation."

Other parasites have been associated with the lesions in cyprinids and other fish. Thus, Plehn (1924) reported that the fish louse, the copepod *Argulus*, occurred frequently on the diseased fish, while Smith (1935) showed that winter flounders with hyperplastic epidermal disease were infected with the metacercarial larvae of the digenetic trematode *Cryptocotyle lingua*. In the diseased bluegill sunfish the ciliates *Trichodina* and *Ichthyophthirius* were present in the overgrowths.

Plehn (1924) believed bacteria to be the primary agent responsible for the hyperplasia, as interpreted by her statement, "Durch infektion gesunder Fische mit Karpfenläusen von den kranken konnten solche Wundpocken experimentell hervorgerufen werden. Da der stich des *Argulus* gewöhnlich diese Wirkung nicht hat, liegt die Annahme nahe, dass der Parasit nur als Uebertrager einer anderen Infektion wirkt; vielleicht sind es Bakterien, die die Hautwucherung veranlassen." It is also possible that *Argulus* and other parasites encountered in fishes showing these lesions may be the vector for a virus. A viral agent was indicated by Loewenthal (1907), who found inclusion bodies in the epithelial cells of diseased European cyprinids. The possibility that *Argulus* transmitted a virus was discussed by Thomas (1931), who attributed this suggestion to Plehn. Smith (1940) considers the disease to be caused by a virus on the basis of Loewenthal's findings. That parasites can and do transmit viral disease is known for higher animals, e.g. swine influenza and salmon poisoning in dogs and men. The former is transmitted by the larvae, which occur in earthworms, of the nematode *Metastrongylus elongatus;* and the latter by the metacercarial larvae, which encyst in the flesh of salmon, of the digenetic fluke *Troglotrema salmonicola.*

It should be pointed out that ectoparasites are found frequently on fishes (Nigrelli, 1943) and in many instances a simple thickening of the skin (corium and/or epithelium) is involved. This is particularly so in the case of infections with *Ichthyophthirius*, a parasite of world-wide distribution. These Protozoa obtain nourishment by feeding on the host tissue cells. Fishes are susceptible to them during periods of varying temperature, which probably accounts for their presence in large numbers, at least in the temperate zone, during the spring. Under these temperature conditions the parasites enter the skin and may be overgrown with epithelium. Whether or not the simple hyperplasia of the epithelium often noted in these infections takes place at the time of active feeding or during the encystment process is not known. It is well known, however, that many parasites are capable of inducing the development of host fibrous tissues, especially during their process of encystment, and it is also probable that a hyperplasia of epithelial tissue may be another manifestation of this response, as in the case of papillomatous diseases associated with parasites (Nigrelli and Smith, 1940).

Schäperclaus (1935) suggested that the hyperplastic epidermal growth occurring in European cyprinids was a manifestation of a nutritional deficiency, but gave no evidence to support this. Nevertheless, this hypothesis finds some support in the work of Wolf (1945) on the "gill disease" of trout. This disease was manifest as a hyperplasia of the epithelium of the gills of trout fingerlings. Wolf considered this hyperplasia a response to chemical irritants, fish waste products, present in hatchery waters. He further pointed out that the susceptibility to the irritants was increased in those fish kept on a pantothenic acid deficient diet. A similar process may be involved in the epidermal hyperplasia of the bluegill sunfish and in other fishes in which this disease has been reported.

One consistent feature of the hyperplastic epidermal disease of fishes is the presence of parasites. Bacterial, protozoan, helminthic or arthropod parasites have been reported in all cases. Only in one instance (Loewenthal, 1907) were inclusion bodies described. Also, with one exception (Plehn, 1924), all attempts to transfer the disease to normal fish gave negative results. The infective agent in this exceptional case was bacterial, but the evidence was not conclusive. It may be concluded, then, that the epidermal hyperplasia found in fishes, is the response of sus-

ceptible hosts to toxic or mechanical irritants produced by parasites.

SUMMARY.

A hyperplastic epidermal disease, similar to "carp-pox," is described in bluegill sunfish, *Lepomis macrochirus*. The disease was endemic in a lake near New Preston, Connecticut, for the four consecutive years from 1938-1941. During this period 200 diseased fish were collected and studied. The lesion is characterized by papillomatous-like nodules or patches and histologically consists of an extensive hyperplasia of epithelial cells and other epidermal elements supported by a delicate stroma. Parasitic infection of the skin with the ciliates *Trichodina* and *Ichthyophthirius* is present, the latter embedded deep in the epithelium. All attempts to induce the disease in normal fish from this and other localities were negative. The disease is a benign regressive hyperplasia, since no invasion of the underlying structures or metastases were found.

The possible role of parasites, chemical irritants and dietary deficiencies in the production of this and similar hyperplastic epidermal growths in fishes is discussed.

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EXPLANATION OF THE PLATES.

PLATE I.

FIG. 1. Bluegill sunfish, *Lepomis machro-chirus*, with epidermal hyperplastic mucoid-like patches. Such extensive growths are present especially in the spring. Slightly less than natural size.

FIG. 2. Fish with patches in the dorso-lateral region, the usual position of these abnormal growths. Such fish are caught frequently during the summer months. Slightly less than natural size.

PLATE II.

FIG. 3. Hyperplastic epidermal growth, showing papillomatous-like arrangement. About 3 ×.

FIG. 4. Isolated scales showing the nature of the overgrowth. Note that each scale's nodule is discrete. About 7 ×.

PLATE III.

FIG. 5. Scale mounted *in toto*. Note the distribution of melanophores, extension of the hyperplastic growth along pathways formed by the scale rings and needle-like crystals interspersed throughout. n, needle-like crystals; e, extension of growth. Hematoxylin-eosin. 75 ×.

FIG. 6. Hyperplastic epidermis with stroma penetrating from the corium. This is a section of growth taken from a fish caught in the spring. Note the orderly arrangement of the cells and lack of any degenerative changes. c, corium; s, scale. Hematoxylin-eosin. 75 ×.

PLATE IV.

FIG. 7. Section showing the upfolding of the growth. Note the cellular arrangement and thickened basement membrane of the columnar cells. The corium is slightly edematous. c, corium; s, scale; bm, basement membrane. Hematoxylin-eosin. 80 ×.

FIG. 8. Section of nodule. The light vacuolated bodies are mucus cells. Giemsa. 75 ×.

PLATE V.

FIG. 9. Epithelial cells as elongate, fibroblastic-like elements, growing out from the base of the growth. Note the mass of blood elements on the right. Hematoxylin-eosin. 675 ×.

FIG. 10. Details of the growth shown in Fig. 6. From the corium in the lower left, the columnar epithelial cells radiate out and become polyhedral and flattened elements towards the periphery. Most of the nuclei are in the pro-chromosomal stage, but some mitotic figures can be seen. Hematoxylin-eosin. 675 ×.

PLATE VI.

FIG. 11. Follicle-like arrangement of mucus cells. Masson. About 1,000 ×.

FIG. 12. Cyst-like body formed by epithelial cells. Hematoxylin-eosin. 1,000 ×.

PLATE VII.

FIG. 13. A single *Ichthyophthirius* embedded deep within the hyperplastic epithelium. Note the numerous ingested epithelial cells. Masson. 900 ×.

FIG. 14. *Trichodina* on the surface of the growth. Note the degeneration of peripheral cells, pyknotic nuclei and sloughing. Hematoxylin-eosin. 675 ×.